# Roles of odor, taste, and toxicity in the food preferences of lambs: implications for mimicry in plants

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In the traditional sense, food ingestion consists of prehending, masticating, swallowing, and digesting plant matter. It is also possible to ingest plants without eating them. Volatile compounds are inhaled directly into the lungs and transported from the lungs into the bloodstream. Volatiles in high concentrations could presumably produce toxicosis, without an herbivore ever ingesting a plant in the customary sense. Volatile compounds may be aposematic, serving to warn potential predators of toxins in plants. We conducted three experiments to explore the roles of odor, taste, and toxicity in the food preferences of lambs. The first experiment determined if brief exposure to a novel odor followed by lithium chloride (LiCl)-induced toxicosis caused lambs to avoid a familiar food that contained the odor. Lambs that sniffed coconut-flavored barley and then received LiCl subsequently ate less coconut-flavored barley than lambs that did not receive LiCl. The second experiment determined if lambs were deterred from eating a familiar food by the odor of *Astragalus bisulcatus*. *A. bisulcatus* is a malodorous (to humans) sulfur-containing herb considered unpalatable and toxic. Neither odor nor intraruminal infusions of *A. bisulcatus* deterred lambs from feeding. The third experiment also determined how the degree of familiarity with the odor of *A. bisulcatus*, along with toxicosis, influenced preference of lambs for food with or without the odor of *A. bisulcatus*. Lambs with 8 d exposure to the odor but not given LiCl ate similar amounts of food, with and without the odor of *A. bisulcatus*, whereas lambs given LiCl showed a mild aversion to food with the odor during testing. Lambs with 1 d exposure to the odor but no LiCl ate similar amounts of food, with and without the odor, whereas lambs given LiCl showed a strong but transient aversion to food with the odor. Collectively, these findings show that lambs responded strongly to novel odors, but their response was transient and depended on the postingestive consequences of toxins and nutrients associated with odor inhalation. Thus, we submit that odor alone, in the absence of toxicosis or nociception, is not a deterrent to herbivores that continually sample foods and adjust intake based on the postingestive effects of toxins and nutrients. It also is unlikely that non-toxic plants can mimic the odors of toxic plants to avoid herbivory (Batesian mimicry), unless the odors are indistinguishable by herbivores, again because herbivores constantly sample foods.

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Plants are composed of a diverse mix of chemicals. Some stimulate and others deter feeding. Mammalian herbivores experience these phytochemicals through the senses of smell and taste in play with the postingestive effects of nutrients and toxins (Provenza 1995a, b).

Herbivores can ingest plants in either of two ways. In the traditional sense, ingestion consists of prehending, masticating, swallowing, and digesting plant matter. It is possible, however, to ingest plants without eating them. Volatile compounds, for instance, are inhaled directly into the lungs and transported from the lungs into the bloodstream. Volatiles in high concentrations can produce toxicosis without an herbivore ever ingesting a plant in the customary sense. Plants could in theory avoid herbivory if they were able to produce high enough concentrations of volatiles to cause toxicity by inhalation. Conversely, volatile plant compounds need not be toxic to offer protection from herbivory if non-volatile compounds in the plant are toxic. In that case, an experienced herbivore would be warned by odor to avoid the plant. Such "olfactory aposematism" may serve as a primary warning system signaling the potentially deleterious effects of toxic plants (Eisner and Grant 1980). Pre-ingestive odor cues may be particularly beneficial for herbivores when non-volatile toxins are poisonous in small doses, or when the ingested toxin has delayed post-ingestive effects. Pre-ingestive odor cues can shorten foraging decision times and reduce the risks associated with poisoning (Augner and Bernays 1998).

Considerable research has been done to understand ingestion in the traditional sense, but there has been little effort to discern the role of volatile compounds in food selection by mammalian herbivores. Even on causal observation, it is apparent that the sense of smell plays a vital role in discrimination and generalization: foraging is an ongoing process of sniffing, avoiding, ingesting. Nevertheless, to our knowledge there are no documented demonstrations that volatile plant compounds per se actually deter feeding by mammalian herbivores, either through their direct harmful effects on olfactory or gustatory receptors (i.e., nociceptive effects) or by producing toxicosis. The scarcity of demonstrated effects may be due to a lack of inquiry or to a lack of deterrence by volatile compounds in concentrations plants produce.

Our objective was to explore the roles of odor, taste, and toxicity in the food preferences of lambs. We conducted three experiments. The first experiment determined if brief exposure ( < 60 s) to a novel odor, followed by toxicosis, caused lambs to avoid a familiar food that contained the odor. The second experiment determined if lambs were deterred by the odor of *Astragalus bisulcatus*, a potentially toxic plant that produces volatile compounds. The third experiment determined how degree of familiarity with the odor of *A. bisulcatus*, along with toxicosis, influenced preference of lambs for food with or without the odor of *A. bisulcatus*. In contrast to the first experiment, lambs in the second and third experiments inhaled the odor while eating, but did not ingest the source (i.e., *A. bisulcatus*) of the odor.

# Experiment 1

## Materials and methods

The objective of Experiment 1 was to determine if brief exposure to a novel odor (coconut), followed by lithium chloride (LiCl)-induced toxicosis, caused lambs to avoid a familiar food (barley). In this experiment, the coconut flavor was mixed with barley during exposure and testing.

Thirty-two lambs (white-faced, commercial crossbreds, 5 mo age, 42 kg BW) were assigned randomly to four treatments in a factorial design that included flavor (3 or 6% coconut-flavored barley) and oral intubation with LiCl (yes or no). Lambs were not familiar with coconut, but they had eaten barley since 1 mo age. During the experiment, lambs were housed individually in adjacent pens.

On d 1, lambs were offered 500 g of ground barley grain for 10 min. We determined intake for each lamb, and used the value as a base to compare with intake post-LiCl. On d 2, lambs were exposed to coconut-flavored barley in plastic containers, but the food was covered with hardware cloth (13 × 13 mm mesh) so lambs could sniff but not eat. Lambs were exposed for 60 s, and we recorded with stop watches the amount of time each lamb spent with its nose in the food box. Immediately after the 60-s exposure, lambs received by oral intubation 200 ml of a solution containing 250 mg/kg BW of LiCl.

On d 3 to 5, we offered lambs 500 g of coconut-flavored ground barley from 0800 to 0810, and then determined intake. Lambs had access to alfalfa pellets from 0900 to 1700 daily, but they were fasted overnight. Lambs had ad libitum access to trace-mineral salt blocks and water.

## Statistical analysis

The repeated measures analysis of variance for food intake was a factorial with odor (3 vs 6%) and LiCl (yes or no) as the main effects; lambs/odor × LiCl was the error term for odor, LiCl, and the odor × LiCl interaction. Day (n = 4) was the repeated measure; lambs/odor × day was the error term for day and its interactions with odor and LiCl.

## Results

Lambs in all groups ate similar amounts of unflavored barley on d 1, before conditioning with LiCl (Fig. 1, d 1). On d 2, just before conditioning with LiCl, the average lamb spent 32 s with its nose in the food box, presumably sniffing coconut-flavored barley. There were no differences for lambs in the different odor-LiCl treatments in time with nose in the box. On d 3 to 5,

lambs that received LiCl ate less coconut-flavored barley than lambs that did not receive LiCl ($P = 0.0005$), and the differences persisted throughout testing ($P = 0.0001$; Fig. 1, d 3 to 5). Lambs that did not receive LiCl decreased intake of coconut-flavored barley on d 3, but they were eating baseline levels of barley by d 4 (Fig. 1). Concentration of odor (3% or 6%) and associated interactions did not influence intake ($P = 0.221$).

## Experiment 2

This experiment determined if the odor of *Astragalus bisulcatus* was aposematic of plant toxicity for lambs naive to *A. bisulcatus*. *A. bisulcatus* is a malodorous (to humans) sulfur-containing herb considered unpalatable and toxic. It is thought to be an obligate selenium-absorbing plant because it is found only in highly seleniferous soils and it sequesters selenium compounds. Volatile sulfur odors can deter feeding (Mason et al. 1994), and it has been proposed that herbivore avoidance of sulfur odors results from either the association of sulfur compounds with predator odors (Nolte et al. 1994) or the association of sulfur with toxic selenium compounds (Mason et al. 1999). Recent studies have linked some of the toxic effects of *A. bisulcatus* to selenium (Panter et al. 1996). Selenium poisoning occurs primarily when animals are stressed (James et al. 1992). Commercial losses to acute selenium poisoning have been estimated to approach $10 million annually (Mayland 1995).

This experiment had two objectives: (1) to determine if lambs were deterred from eating by the odor of *A. bisulcatus*, and (2) to examine the association between odor, *A. bisulcatus*-induced toxicity, and food intake. In both trials, lambs were offered only one food during testing. In contrast to Experiment 1, lambs inhaled the



Fig. 1. Intake (means ± standard errors) of coconut-flavored barley by lambs. Baseline intake of barley was determined on d 1. Lambs were allowed to sniff, but not eat, coconut-flavored barley on d 2, and then half of them were given LiCl. Lambs were offered coconut-flavored barley on d 3 to 5 ($LSD_{0.05} = 44$ g).

Table 1. Composition of artificial odor solution in water used to deliver the volatiles of green *Astragalus bisulcatus*.

| Chemical component | Concentration (ppm) |
| --- | --- |
| Acetaldehyde | 10.1 |
| Ethanol | 10.5 |
| Pentanal | 0.03 |
| Hexanal | 0.03 |
| 2-Hexenal (trans) | 0.008 |
| Heptanal | 0.02 |
| Octanal | 0.03 |
| Methyl sulfide | 0.008 |
| Methyl selenide | 0.008 |
| Dimethyl disulfide | 0.008 |
| Dimethyl diselenide | 0.008 |

odor while eating, but did not ingest the source of the odor (i.e., *A. bisulcatus*).

## Materials and methods

### Odor delivery

*A. bisulcatus* (4 kg) was collected near Kaycee, WY, USA, and air dried for 4 d before homogenization with a Robot Coupe RSI 6V commercial food processor (Robot Coupe, Jackson, MS, USA) at 3000 rpm for 10 min. Plant material used as an odor source was not subjected to any further treatment. Plant material delivered to the test animals via oral intubation was finely ground using a Wiley mill equipped with a 1-mm sieve. The dried plant material was analyzed for total selenium content by a contract laboratory (Huffman Laboratories, Inc., Golden, CO, USA).

To reliably deliver the odor of green plant material, an artificially prepared odor solution was produced to mimic the volatile composition of freshly cut *A. bisulcatus* (Table 1). Both the artificial odor and dried *A. bisulcatus* were used in Experiment 2. The volatile profiles of dried and freshly cut plant material as well as the artificial odor were determined by purge and trap gas chromatography. A Tekmar 3000 purge and trap concentrator (Tekmar, Cincinnati, OH, USA), equipped with a Supelco Vocarb 3000 trap (Supelco, Bellefont, PA, USA), was used to purge 1 g plant samples with helium for 10 min. Volatiles were thermally desorbed onto a Hewlett-Packard 5890 gas chromatograph (Hewlett-Packard Co., Avondale, PA, USA) equipped with a 30 m × 0.2 mm fused silica capillary column (DB-5, J & W Scientific, Folsom, CA, USA) and a Hewlett-Packard 5972 mass selective detector. Volatiles were desorbed at 250°C onto the injection port of the gas chromatograph that was set at 200°C. The initial oven temperature of 0°C was maintained for 5 min until elevated to 150°C at a rate of 20°C/min where it was maintained for 2.5 min. Volatile compounds were identified by their mass spectra.
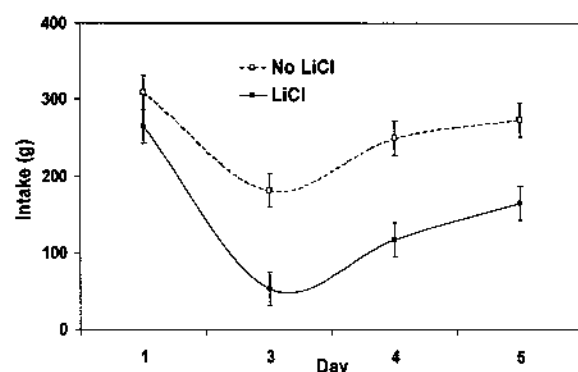
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The artificial odor solution was incorporated into a cross-linked polymer to facilitate delivery during the feeding trials. An aqueous polymer solution was made with a polyvinyl alcohol (avg MW 130000) solution at a concentration of 40 g/L and a separate cross-linking solution made with 40 g of sodium tetraborate dodecahydrate in 1.0 L of water. Each odor delivery vehicle was made by first adding 100 μL of the artificial odor solution to 10.0 mL of the polyvinyl alcohol polymer solution. After brief mixing, 2 mL of the cross-linking solution was added and the solution was mixed until a cross-linked polymer with the consistency of gelatin was produced.

Polymeric odor delivery vehicles were placed in coffee filters and closed with staples for presentation to the lambs. Dried plant material odor delivery vehicles were similarly prepared by placing approximately 5 g of dried A. bisulcatus in coffee filters. Control odor delivery vehicles were prepared using approximately 5 g of the barley-straw test mixture. Polymeric odor delivery vehicles were prepared fresh daily; dried plant and control delivery vehicles were re-used for 3 d successively. Plastic food containers (sides of 13 cm) were equipped with 6 cm (H) × 8 cm (W) × 1.5 cm (D) "cages" made of hardware cloth to hold the filter papers. Cages were affixed near the top of one side of each food container and held in place by elements of the cloth itself that were passed through 1.5-mm holes in the container.

*Exposure*
A group of 30 lambs (crossbreds, 5 mo age, 40 kg BW), different from those used in experiment one, were offered 400 g of a ground barley grain:ground wheat straw (70:30 by weight) test mixture in plastic containers equipped with the odor delivery system. All lambs were fed the barley-straw mix without odors for 2 d to familiarize them with the feeding regime. Intake data from this adaptation period were used to assign lambs into three groups (10/group) such that consumption was balanced among the groups. Lambs in group 1 were exposed to the artificial A. bisulcatus odor, those in group 2 were exposed to the dried A. bisulcatus odor, and lambs in the third group (control) were exposed to the control odor. The odor delivery vehicle was presented in a frontal and distal position relative to the animal.

Single-choice feeding trials were conducted daily from 0830 to 0900 for 7 d. Lambs were exposed to the odor of A. bisulcatus, without oral gavage of A. bisulcatus or LiCl, on d 1 to 7. Plant infusions were delivered by oral gavage immediately after the 30-min feeding trials on d 7 and 8. After each trial, intake was recorded and alfalfa pellets were offered ad libitum until 1700. The individually penned animals were

provided ad libitum access to water and trace-mineral salt blocks throughout the study.

On d 7, lambs in treatments 1 and 2 were infused with A. bisulcatus by oral intubation at a rate of 0.2 g plant material per kg BW just after the feeding trial. Dried and finely ground plant material was delivered as a slurry in 100 mL tap water. Lambs in the control odor group were infused with 100 mL tap water. Infusions were repeated on d 8 except that the amount of A. bisulcatus was increased to 0.4 g per kg BW. Access to the basal ration of alfalfa pellets was delayed 30 min following plant infusions.

## Statistical analyses

We conducted two separate analyses of variance. The ability of A. bisulcatus odors to deter feeding was investigated by analyzing the intake data for d 1 to 7 with a repeated measures analysis. Treatment was the main effect and day the repeated measures. Lambs/treatment was the error term for treatment and lamb/treatment × day was the error term for day and the interaction of day and treatment. Intake data for d 8 and 9 were analyzed separately to examine the roles of plant ingestion and odor. The model for this analysis was similar to the first.

## Results

Intake of barley-straw varied by treatment and day from d 1 to d 3 ($P = 0.002$; Fig. 2). However, treatment differences were not maintained after d 4 (treatment effect $P = 0.703$). Neither the artificial odor nor the dried plant odor persistently deterred feeding on the barley-straw mix.
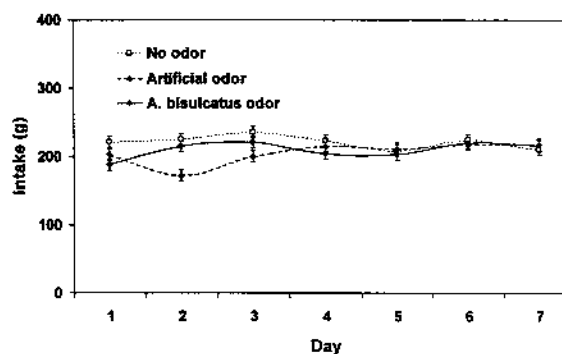


Fig. 2. Intake (means ± standard errors) of a barley-straw mix by lambs in treatment – artificial odor or *Astragalus bisulcatus* odor – and control groups. Odors were placed in the food boxes such that lambs smelled, but could not eat, the source of the odor ($LSD_{0.05} = 16$ g).

Infusion with dried plant material on d 8 (0.2 g/kg BW) and 9 (0.4 g/kg BW) did not influence intake by lambs ($P = 0.793$), and treatment did not interact with day ($P = 0.885$). Lambs in the control group, those exposed to the artificial odor, and those exposed to the odor of A. bisulcatus, did not differ in intake of the barley-straw mix following gavage with 0.2 g (202 vs 195 vs 209 g) or 0.4 g (230 vs 221 vs 231 g) per kg BW. Lambs were infused with 12 mg of selenium on d 7 and with 24 mg on d 8.

## Experiment 3

The objective of this experiment was to determine how familiarity with the odor of A. bisulcatus and LiCl-induced toxicosis influenced preference of lambs for food in boxes with or without the odor of A. bisulcatus. Similar to Experiment 2, lambs inhaled the odor of A. bisulcatus while eating, but did not ingest the source of the odor. In contrast to Experiments 1 and 2, lambs could choose between food with or without the odor of A. bisulcatus.

## Materials and methods

A group of 30 lambs (crossbreds, 5 mo age, 31 kg BW), different from those used in Experiments 1 and 2, were allotted to two groups. Half of the lambs were exposed to the odor of A. bisulcatus while feeding, whereas the other half were not. All lambs were fed a mix of ground barley grain-ground wheat straw (70:30 by weight) in a plastic container.

Lambs were exposed to the odor of A. bisulcatus while eating from 0800 to 0830 for 7 d. Lambs were offered 250 g of the barley-straw mix on the first 3 d, and 300 g on the last 4 d. Intake of the barley-straw mix was recorded each day at 0830, and alfalfa pellets were fed ad libitum until 1700. Lambs were without food from 1700 until 0800 the next day. During the study, lambs were penned individually with access to trace-mineral salt blocks and water. Alfalfa pellets were the basal diet.

After the 7-d exposure, lambs were sorted by the amount of barley-straw ingested during the last 4 d of exposure. Pairs of lambs were randomly assigned to two additional groups, LiCl or no LiCl, such that intake of the barley-straw mix was balanced between groups. Sixteen lambs were assigned to the no-LiCl group and 14 lambs to the LiCl group. Lambs in each group were exposed to the plant odor on d 8. There were four treatments: (1) 8-d exposure to the odor of A. bisulcatus, no LiCl; (2) 8-d exposure to the odor of A. bisulcatus, plus LiCl; (3) 1-d exposure to the odor of A. bisulcatus, no LiCl; and (4) 1-d exposure to the odor of A. bisulcatus, plus LiCl.
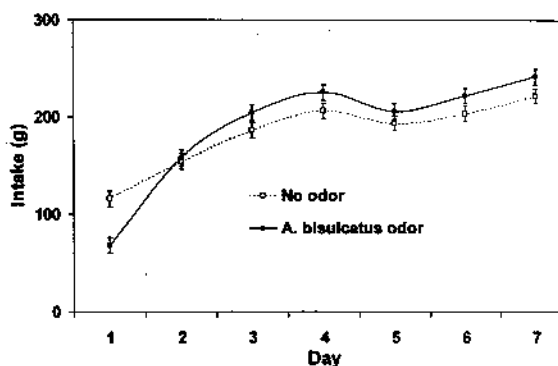


Fig. 3. Intake (means ± standard errors) of a barley-straw mix by lambs with or without the odor of Astragalus bisulcatus in their food boxes. Odors were placed in the food boxes such that lambs smelled, but could not eat, the source of the odor ($LSD_{0.05} = 16$ g).

After being assigned to groups, all lambs were fed 300 g of the barley-straw mix with the odor of A. bisulcatus from 0800 to 0830 on d 8. At 0830, lambs in the LiCl group received by oral intubation 200 ml of a solution containing 150 mg/kg BW of LiCl. For 5 d following the infusion with LiCl, all lambs were fed the barley-straw mix in two food boxes from 0800 to 0830. One box contained the barley-straw mix with the odor of dried A. bisulcatus, whereas the other box contained the barley-straw mix without the odor (i.e., the filter paper bag with only the barley-straw mix). Alfalfa pellets were fed ad libitum from 0830 to 1700. Lambs were without food from 1700 until 0800 the next day.

## Statistical analyses

We conducted three analyses of intake data, one for the 7 d of exposure, another for the day lambs received LiCl, and a third for the 5 d of preference tests. The first analysis (exposure) was a repeated measures with group as the main effect and lambs/group as the error term; day ($n = 7$) was the repeated measure. The second analysis (LiCl) was similar to the first, except there was no repeated measure. The third analysis (test) was a split-plot with days of exposure to the odor (1 or 8) and LiCl (yes or no) as the whole-plot; preference for food (with or without the odor of A. bisulcatus) was the sub-plot; day was the repeated measure. Lamb/odor × LiCl was the error term for the whole-plot. Lamb/odor × LiCl × preference was the error term for the sub-plot. Lamb/odor × LiCl × preference × day was the error term for day and its interactions with odor, LiCl, and preference.

## Results

On the first day of exposure, lambs ate less of the barley-straw mix when the food box contained the odor of *A. bisulcatus*, but for the next 6 d there were no differences between treatments (group × day interaction, $P = 0.0001$; Fig. 3). Lambs in both treatments generally increased intake of the barley-straw mix throughout the 7-d exposure ($P = 0.0001$).

On d 8, when lambs received LiCl, half of the lambs were exposed to the odor of *A. bisulcatus* for the first time. Again, lambs unfamiliar with the odor ate about half as much of the barley-straw mix as lambs familiar with the odor (119 vs 222 g; $P = 0.0001$).

On d 11 to 15, when lambs were offered a choice of the barley-straw mix with or without the odor of *A. bisulcatus*, there was an interaction between previous exposure to the odor, LiCl, and day ($P = 0.0001$; Fig. 4). Lambs with 8-d exposure to the odor – but not given LiCl–ate similar amounts of barley-straw mix, with or without the odor of *A. bisulcatus* (Fig. 4a); lambs given LiCl showed a mild aversion to food with the odor (Fig. 4b). Lambs with 1-d exposure to the odor – but no LiCl–ate similar amounts of barley-straw mix, with or without the odor (Fig. 4c); lambs given LiCl showed a strong but transient aversion to food with the odor during testing (Fig. 4d).

## Discussion

### Olfactory aposematism and odor aversions

Olfactory aposematism may be a way that plants warn herbivores. Rothschild (1964) suggested that chemically defended insects share a common odor, and one class of compounds (pyrazines) is common among noxious or distasteful insect prey (Rothschild and Moore 1987). Domestic chicks readily associate pyrazine odors with deleterious or distasteful prey (Guilford et al. 1987). The odor of pyrazines also potentiates an association between environmental cues and unpalatable taste in rats (Kaye et al. 1989). However, little research has been done to determine if mammalian herbivores use olfactory aposematism to avoid noxious or distasteful forages, as suggested by Eisner and Grant (1980), or if odor inhalation alone can deter herbivores from foraging on particular plants.

Lambs avoided a novel odor (coconut), even with brief exposure (32 s), when toxicosis followed odor inhalation (Fig. 1). Lambs in both groups – LiCl or no LiCl – decreased intake on the first day they were offered coconut-flavored barley. The decline in intake persisted for only 1 d in controls, whereas lambs that received LiCl ate less coconut-flavored barley for 3 d after exposure to LiCl. The addition of a novel flavor to
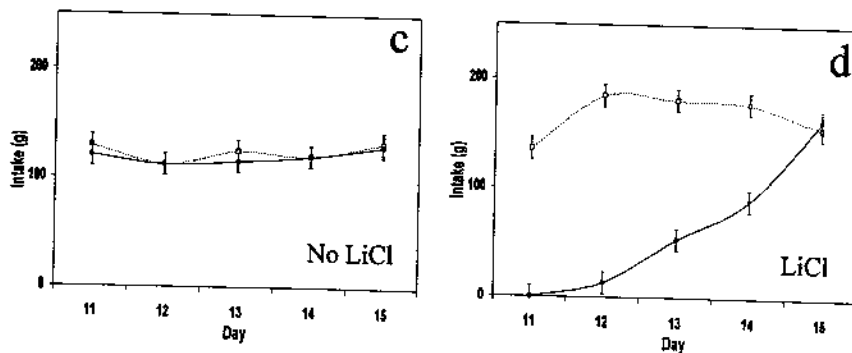
## 8 d Exposure to Odor
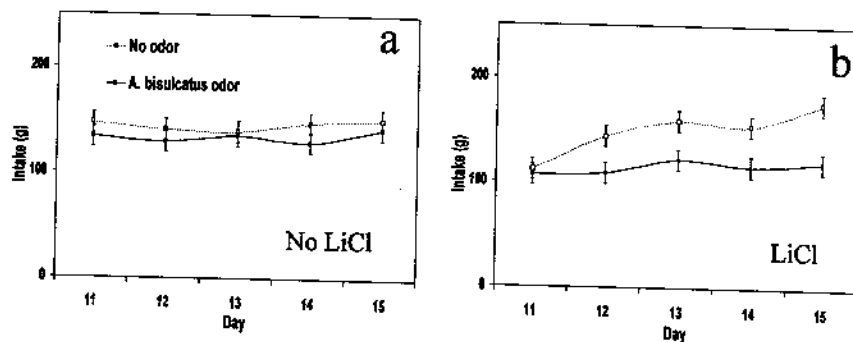


## 1 d Exposure to Odor



Fig. 4. Intake (means ± standard errors) of a barley-straw mix, with or without the odor of *Astragalus bisulcatus*, by lambs that varied in previous exposure to the odor of *A. bisulcatus* (8 d vs 1 d). Half of the lambs in each group received LiCl following exposure to the odor of *A. bisulcatus*, whereas the other half did not. Odors were placed in the food boxes such that lambs smelled, but could not eat, the source of the odor ($LSD_{0.05} = 18$ g).

a familiar food decreases intake (Provenza et al. 1996), and the present results show that the response is amplified when lambs experience toxicosis following odor inhalation.

Though not tested explicitly in our experiments, lambs apparently maintained a stronger aversion when an odor and taste were both present in the food (Experiment 1; Fig. 1), than when only an odor was present (Experiment 3; Fig. 4d) during post-conditioning tests. This finding is consistent with the hypothesis that taste aversions (Palmerino et al. 1979), and taste-potentiated odor aversions (Slotnick et al. 1997), are more resistant to extinction than odor aversions alone (Garcia 1989). It is also consistent with the finding that odor alone is not as effective as the combination of odor and taste in deterring herbivory by sheep (Launchbaugh and Provenza 1993).

We hypothesized that sheep would associate the odor of a potentially toxic plant (*A. bisulcatus*) with the aversive consequences of eating the plant. However, ingestion (oral intubation) of *A. bisulcatus* in moderate amounts did not deter feeding by sheep. Either the amount of toxin delivered was insufficient to produce gastrointestinal distress, or the lambs were not particularly sensitive to toxicosis caused by selenium or the neurotoxin swainsonine (Molyneux et al. 1989). Pigs exhibit severe signs of selenium toxicosis within 5 d when fed *A. bisulcatus* (25 mg selenium/d; Panter et al. 1996), but *A. bisulcatus*-induced toxicosis may not manifest at the same rate or degree in sheep as in pigs (Panter et al. 1995).

Lambs consistently ate less from the barley-straw food box with the odor of *A. bisulcatus* on d 1 of exposure, but intake increased quickly, typically by d 2 of exposure (e.g., Fig. 3). Even when offered a choice (Experiment 3), lambs ate similar amounts of barley-straw with or without the odor. Indeed, lambs that received LiCl after only 1 d of exposure to *A. bisulcatus* ate equally from food boxes with or without the odor after 5 d (Fig. 4d). Collectively, these data suggest that intake of barley-straw was not constrained by lack of alternatives but by lack of aversiveness of the odor.

The cost of producing volatile secondary metabolites in concentrations and amounts that could potentially intoxicate an herbivore merely by inhalation is likely to be high (Gershenzon 1994), particularly for the odor to be effective against large mammalian herbivores. Plants can also reduce the risk of attack, but at a much lower cost, by repelling herbivores through signaling the presence of toxins, and some contend that risk-averse foraging strategies involve the avoidance of strong cues detected through the senses of sight and smell (Augner et al. 1998, Leimar and Tuomi 1998). For this strategy to be effective, there must be a strong correlation between signal and defense. Strong signals are deterrent only when they are correlated with some aversive quality – nociception or toxicosis – of the plant (Rhoades

1979). In our study, the odor of *A. bisulcatus* alone was not aversive. Rather, odors were avoided only when they were novel or after LiCl-induced toxicosis. Repeated exposure to the same food, with or without odor cues, consistently led to extinction of the avoidance response (Figs 2–4).

Avoidance of the barley-straw mix increased following toxicosis, and the response was most pronounced when lambs were naive to the odor of *A. bisulcatus* (Fig. 4). Strong avoidance of novel foods also is evident when animals eat familiar and novel foods and subsequently experience toxicosis: they avoid the novel foods, not the familiar foods, in ensuing meals (Burritt and Provenza 1989, 1991). When they become ill after a meal of novel foods, they avoid the foods whose flavors are most different from familiar foods (Kalat 1974, Burritt and Provenza 1989, Launchbaugh et al. 1993, Provenza et al. 1994). Strong combinations of flavor-feedback signals on first consumption of a plant most effectively deter foraging by herbivores such as lambs.

## Dynamics of preference

Some suggest that Batesian mimicry, in which a predator avoids attacking edible prey that appears similar to noxious prey, could be important in plant-herbivore interactions (Rhoades 1979, Launchbaugh and Provenza 1993). Some modeling efforts also imply that mimicry may be common (Augner and Bernays 1998). Nevertheless, there are no studies to show conclusively that Batesian mimicry is prevalent in terrestrial plant-herbivore systems. Nor has there been much work to determine whether or not herbivores might be deceived by mimics (Augner and Bernays 1998).

Plants should signal defenses to deter herbivores (Augner 1994), and herbivores generalize aversions from defended to undefended plants (Launchbaugh and Provenza 1993), so mimicry is possible. Herbivores also are sensitive to the intensity of the defense signal, so stronger signals can further enhance deterrence (Launchbaugh et al. 1993). Lambs prefer familiar foods with low as opposed to high concentrations of added flavors (e.g., onion) when the nutrient content of the food containing the flavors is constant (Augner et al. 1998). Thus, salient flavors can deter feeding under certain conditions.

Nonetheless, herbivores constantly sample plants, and dynamic relationships continually emanate between behaviors and consequences – flavor-postingestive feedback loops (Provenza 1996, Provenza et al. 1998). Flavor-feedback interactions increase the likelihood of plant ingestion when the postingestive consequences are positive, and they decrease the probability of ingestion when the consequences are aversive. The inherently dynamic and adaptive nature of flavor-feedback interactions makes it unlikely a plant can long avoid her-

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bivory if it produces a defense signal without the presence of a defense. Predictions from game theory also suggest that if herbivores continually sample plants, then mimicry is unlikely to evolve (Augner and Bernays 1998). Mammalian herbivores also possess keen senses (odor, taste, sight) that enable them to discriminate among similar flavor signals with different postingestive consequences. Goats discriminate, even among similar parts within the same plant (Provenza et al. 1983, 1994). Thus, a mimic's signal must be virtually identical to that it mimics to avoid herbivory.

Our findings suggest that while lambs responded strongly to novel odors, the reaction was transient and depended on the postingestive effects of odor inhalation. In the absence of aversive consequences, odor inhalation did not produce lasting deterrence (Figs 3, 4). When the odor was novel and the consequences were aversive, lambs were deterred for longer periods than when the odor was merely novel, but even then the aversion was transient (e.g., Fig. 4d). Conversely, lambs learn quickly to eat foods with high concentrations of flavors when the consequences of food ingestion are positive (Villalba and Provenza 1996, 1997a, b, c, 1999, Wang and Provenza 1997). Lambs exposed to low or high concentrations of added flavors, and then given low or high amounts of energy (starch) by intraruminal infusion, learn to prefer either flavor concentration, depending on whether low or high amounts of energy are infused after they eat straw with different flavor concentrations (Villalba and Provenza unpubl.). Thus, strong odor signals may even be detrimental for undefended plants with high concentration of nutrients and little or no toxins.

## Conclusion

Our results suggest that odors are deterrent when they are paired with toxicosis. Avoidance of a salient cue – the odor of *A. bisulcatus* – was transient (1 d), due to novelty and not to the negative impact of the odor. Toxicosis following inhalation of a novel odor effectively conditioned an aversion to a familiar, nutritious food, but the response was transient (5 d) when the toxin was no longer administered. This aposematic role of odor offers protection to plants without having to be frequently eaten. However, the ephemeral nature of the avoidance suggests that ongoing toxicosis is necessary for prolonged protection (Bryant et al. 1991). Thus, we submit that odor alone, in the absence of toxicosis or nociception, is not a deterrent to herbivores that continually sample foods and adjust intake based on the postingestive effects of toxins and nutrients. It also is unlikely that non-toxic plants can mimic the odors of toxic plants (Batesian mimicry) to avoid herbivory (Eisner and Grant 1980, Launchbaugh and Provenza 1993), unless the odors are indistinguishable by herbivores, again because herbivores constantly sample foods.

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